REMARKS

Status of the Claims

Claims 1-11 are pending in this application. No claims have been canceled, added or amended. Applicants submit the following arguments in support of the allowability of the claims. Applicants respectfully request that the Examiner enter this Reply as no new issues have been raised.

Priority

Contrary to the Examiner's statements in the Office Action, an Applicant need only provide a certified copy of the originally filed foreign application. Such a copy has been filed. No translation is required according to 37 CFR 1.55 and MPEP 201.13. As such, Applicants respectfully request that this objection be withdrawn as improper.

Information Disclosure Statement

Applicants submit that all references cited in the specification were submitted in an Information Disclosure Statement dated July 27, 2001. As such, Applicants respectfully request that this objection be withdrawn as improper.

Rejections under 35 USC 103(a)

The Examiner maintains the rejection of claim 1 as obvious over Yamao USP 6,030,845 in view of the abstract of JP 60047962. Applicants traverse the rejection and respectfully request the withdrawal thereof.

In response to Applicants' arguments in the Reply filed May 27, 2003, the Examiner states that hemolysis may occur before or after the agglutination reaction. The Examiner points to column 2, line 4 of Yamao '845. The Examiner states that the order of lysis is not critical to the invention and that Yamao '845 and JP '962 teach simultaneous lysis and agglutination.

Applicants submit that the present invention is directed to a whole blood immunoassay comprising the steps of (i) mixing a whole blood sample with sensitized insoluble carrier particles to cause an immune agglutination; (ii) diluting the resulting agglutination mixture with an aqueous solution containing an erythrocyte lysing agent to lyse erythrocytes; and (iii) determining the degree of agglutination of the resulting whole blood sample.

Neither Yamao '845 nor JP '962 discloses an immunoassay for whole blood where the whole blood is first subjected to the agglutination reaction and then lysed subsequent to the agglutination reaction. Yamao '845 discloses an immunoassay where the agglutination reaction occurs simultaneously with hemolysis of the whole blood cells as opposed to the reaction occurring before

hemolysis as in the present invention. In fact, Yamao '845 teaches away from first subjecting the whole blood to the agglutination reaction and then lysing subsequent to the agglutination reaction. Yamao '845 teaches that lysis should not be carried out in a manner that would affect agglutination. See column 6, lines 5-8 which disclose that the surface-active agents inhibit the agglutination reaction. Therefore, one of ordinary skill in the art would not be motivated to alter the Yamao '845 assay to first subject the whole blood to the agglutination reaction and then lysis. In addition, in Example 4 of Yamao '845, the agglutination reaction was carried out after lysis, as would be expected from the complete disclosure in the specification in Yamao '845.

JP '962 discloses a whole blood immunoassay where the lysing agent is added to the whole blood and lysis occurs prior to agglutination. In the Office Action, the Examiner stated that hemolyzing agents cause hemolysis of erythrocytes, which interfere with the agglutination reaction by referring to the abstract of JP '962. Applicants respectfully disagree with the Examiner and provide an English translation of the complete specification of JP '962. (A copy is attached hereto.) Upon review of the English translation, Applicants submit that the Examiner's statement is not supported by the entire specification of JP '962. As such, Applicants respectfully submit that the Examiner's statement is not correct and that JP '962 fails to suggest that lysis should occur

after agglutination. As such, the Examiner's reliance on JP '962 is insufficient in combination with Yamao '845 to disclose or suggest all the elements of the present invention.

The Examiner also maintains the rejection of claims 2 and 3 as obvious over Yamao '845 in view of the abstract of JP '962 and further in view of Bester et al. Applicants traverse the rejection and respectfully request the withdrawal thereof.

The Examiner cites Bester for disclosing optimization of the lysing agent, such as SDS. Bester also fails to disclose having agglutination occur prior to lysis. Inasmuch as Bester fails to compensate for the deficiencies in Yamao '845 and JP '962, as pointed out above, Applicants submit that this rejection should also be withdrawn for the reasons stated above.

Claims 4 through 9 are also rejected over Yamao '845 in view of the abstract of JP '962, Kosako USP 5,527,714 and Cohen USP 4,851,329. Applicants traverse the rejection and respectfully request the withdrawal thereof.

The Examiner cites Kosako '714 and Cohen '329 for teaching the step of determining the concentration of particles to have an assay with high sensitivity and specificity. Inasmuch as Kosako '714 and Cohen '329 fail to compensate for the deficiencies in Yamao '845 and JP '962, as pointed out above, Applicants submit that this rejection should also be withdrawn for the reasons stated above.

The rejection of claim 10 is also maintained as obvious over Yamao '845 in view of the abstract of JP '962 and further in view of Holmes USP 4,830,969.

The Examiner relies on Holmes '969 for disclosing the reaction time and temperature. Holmes '969, however, fails to disclose a whole blood immunoassay where agglutination takes place prior to hemolysis. As such, Applicants submit that Holmes '969 also fails to compensate for the deficiencies in the primary and secondary references, Yamao '845 and JP '962, as pointed out above. Therefore, this rejection should be withdrawn.

Conclusion

As Applicants have addressed and overcome all objections and rejections in the Office Action, Applicants respectfully request that the objections and rejections be withdrawn and that the claims be allowed.

Should there be any outstanding matters that need to be resolved in the present application, the Examiner is respectfully requested to contact Kecia Reynolds (Reg. No. 47,021) at the telephone number of the undersigned below, to conduct an interview in an effort to expedite prosecution in connection with the present application.

Application No. 09/915,580

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. §§ 1.16 or 1.17; particularly, extension of time fees.

Respectfully submitted,

BIRCH, STEWART, KOLASCH & BIRCH, LLP

By _____
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MSW/KJR/crt
0397-0431P

Attachment(s)

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(11) Japanese Patent Application Laid-Open No.60-47962

(43) Laid-Open Date: March 15, 1985

(21) Application No.58-154922

(22) Application Date: August 26, 1983

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(74) Agent: Patent Attorney, Kousuke NISHIMURA

(54) [Title of the Invention] Methods for measuring blood antigen or antibody levels and reagents employed for the same

SPECIFICATION

1. Title of the Invention

Methods for measuring blood antigen or antibody levels and reagents employed for the same

2. Claims

(1) A method for measuring the blood level of an antigen or antibody comprising adding a hemolytic agent and an antigen- or antibody sensitization carrier suspension to a whole blood sample and monitoring the aggregation reaction.

(2) A reagent used for measuring the blood level of an antigen or antibody comprising a hemolytic agent and an antigen- or antibody sensitization carrier.

(3) The reagent according to Claim 2 wherein the hemolytic agent is a saponin.

3. Detailed Description of the Invention

I. Background of the Invention

Field of the Invention

The present invention relates to a method for measuring the blood level of an antigen or antibody as well as a reagent employed for the same.

More particularly, the invention relates to a method for measuring the blood level of an antigen or antibody by means of an aggregation reaction based on an immune reaction

as well as a reagent employed for the same.

The invention is utilized in various immunological tests such as the diagnosis of rheumatoid arthritis.

Prior Art and its Problems

A conventional method for measuring an antigen or antibody in a blood on the basis of the aggregation reaction employs as a sample a serum because of the difficulty in a macroscopic assessment of the aggregation when the sample contains red blood cells. However, the preparation of the serum requires a complicated and time-consuming process for example when handling a large number of the samples. It is also required, in preparing the serum, to collect the blood in an amount exceeding the amount required actually in the test.

II. Objective of the Invention

An objective of the invention is to provide a method for measuring the level of an antigen or antibody directly using a while blood without any tiresome process for preparing a serum sample.

Another objective of the invention is to provide a reagent used in the method described above.

For the purpose of achieving such objectives, the invention consists of a method for measuring the blood level

of an antigen or antibody comprising adding a hemolytic agent and an antigen- or antibody sensitization carrier suspension to a whole blood sample and monitoring the aggregation reaction.

Furthermore, the invention consists of a reagent used in the method mentioned above comprising a hemolytic agent and an antigen- or antibody sensitization carrier.

Moreover, the invention consists of the reagent comprising as a hemolytic agent a saponin.

III. Detailed Description of the Invention

A method according to the invention can be conducted by adding a hemolytic agent and an antigen- or antibody sensitization carrier suspension to a sampled whole blood and monitoring the aggregation reaction.

The hemolytic agent employed in the method mentioned above may for example be a saponin or various surfactants. The hemolytic agent may be added to the whole blood prior to the aggregation reaction to hemolyze red blood cells, or may be added at a concentration of about 0.2 to 2% to the antigen- or antibody sensitization carrier suspension for effecting the hemolysis of the red blood cell upon the aggregation reaction. The antigen- or antibody sensitization carrier is not limited particularly and may be any known material such as a latex resin, inorganic

adsorbent, chemically treated immobilized erythrocyte and the like.

The monitoring of the aggregation reaction can be conducted by the law of the art. Thus, a drop of a whole blood was added dropwise onto a glass slide, to which each one drop of a hemolytic agent and an antigen- or antibody sensitization carrier suspension, mixed thoroughly using a wood rod, and spread over an area of about 20 x 25 mm. The glass slide was held by both hands, shaken gently for 1 minutes, and then examined for any aggregation visually. This visual evaluation is not affected by red blood cells, which have already been lyzed.

The invention is further described in the following Examples.

EXAMPLES

(1) Preparation of human gamma-globulin sensitization latex for detecting rheumatoid factors (RF)

A polystyrene latex (particle size: 0.117 „) is suspended in a glycine-sodium chloride buffer solution (pH 8.2) (hereinafter abbreviated as GNB) at the solid content of 2.0%. On the other hand, a human gamma-globulin which has been dialyzed against GNB was dissolved in GNB at the concentration of 10 mg/ml. The both solutions were mixed in the volume ratio of 1:1, and warmed at 50°C for 1 hour. The

resultant solution was washed by centrifugation (17,000 rpm, 10 minutes), combined with GNB containing 0.5% bovine serum albumin and 0.4% saponin to prepare a 0.4% sensitization latex suspension. Under the condition described above, the sensitization protein concentration was 10 to 100 „gN/ml and the latex particle density was 4.53×10^8 particles/ml, allowing for an assumption that approximately 75,000 molecules of gamma-globulin bind to a single latex particle.

(2) Slide aggregation reaction

A drop of the sensitization latex obtained in (1) described above (about 0.02 to 0.03 ml) and a drop of a blood or serum were mixed thoroughly on a glass slide for reaction, spread over an area of about 2 cm in diameter, and subjected to the aggregation reaction. The glass slide was swung back and forth, and examined after 1 minutes for any aggregation reaction as well as the degree, if any, based on the criteria shown below. The results are shown in Table 1.

Positive (+)

Aggregation clots are observed throughout the wet region, with the aggregation being marked macroscopically.

Negative (-)

No aggregation was observed macroscopically.

Judgement impossible (?)

The latex aggregation is unclear.

Positive control

Rheumatoid arthritis (RA) control serum (aggregation titre: 160)

Negative control

Normal healthy human serum

The control serum described above and normal healthy human concentrated red blood cells were reconstituted (hematocrit: 40%) and used as a sample.

Table 1

	Sensitization latex sample	Saponin-supplemented sensitization latex	Saponin-free sensitization latex
Negative control	Normal healthy human serum	-	-
	Whole blood (anticoagulant-free)	-	?
	Whole blood (heparinized)	-	?
	Whole blood (ACD blood)	-	?
Positive control	RA Serum	+	+
	Whole blood (anticoagulant-free)	+	?
	Whole blood (heparinized)	+	?
	Whole blood (ACD blood)	+	?

Based on the results shown in Table 1, the saponin-supplemented sensitization latex test solution enabled a definitive judgement even when using as a sample a whole blood, revealing an excellent specificity.

IV. Typical effects of the invention

According to the invention, a method for measuring the blood level of an antigen or antibody by which the antigen or antibody level can directly be measured using a while blood.

Since the inventive method employs a hemolytic agent to lyze red blood cells, the aggregation can readily be judged to be present or absent even when using a whole blood as a sample. Accordingly, it eliminates the need of preparation of a serum sample which is essential for an aggregation test in a conventional method, whereby simplifying the measurement.

The invention also provides a reagent employed preferably in the measurement described above. Since the inventive reagent contains a hemolytic agent and an antigen- or antibody sensitization carrier, it lyzes red blood cells only by mixing it with a whole blood, whereby allowing the aggregation test to be conducted easily.

PATENT ABSTRACTS OF JAPAN

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(71)Applicant : TERUMO CORP
(72)Inventor : ITO YOSHITAKA

(54) METHOD FOR MEASURING AMOUNT OF ANTIGEN OR ANTIBODY IN BLOOD AND TEST SOLUTION USED THEREIN

(57)Abstract:
PURPOSE: To make it possible to directly measure the amount of an antigen or antibody from whole blood by omitting labor for preparing serum, by adding an antigen or antibody sensitized carrier floated solution of a hemolytic agent to a whole blood specimen, and tracking the agglutination reaction thereof.
CONSTITUTION: Saponin or various surfactants are used as a hemolytic agent. The hemolytic agent may be preliminarily added to whole blood prior to agglutination reaction to dissolve a red corpuscle or preliminarily added to an antigen or antibody sensitized carrier floated solution in a concn. of about 0.2W2% to dissolve the red corpuscle at the time of agglutination reaction. As the antigen or antibody sensitized carrier, a latex resin, an inorg. adsorbent or an immobilized red corpusele treated with chemicals can be used. The tracking of agglutination reaction is performed according to a usual method. That is, one drop of whole blood is dripped on a glass slide and one drop of the hemolytic agent or the antigen or antibody sensitized carrier floated solution is added to said hemolytic agent while both of them are well mixed by a wooden rod and spread in a size of 20 × 25mm. The glass slide is held by both hands to be shaken and, thereafter, the presence of absence of agglutination is judged with the naked eye.

LEGAL STATUS

[Date of request for examination]

[Date of sending the examiner's decision of rejection]

[Kind of final disposal of application other than the examiner's decision of rejection or application converted registration]

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[Date of extinction of right]